THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

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NEW YORK, N. Y. 10022
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FEB 4 1974

Application for Research Grant
(Use extra pages as needed)

Date: 1/28/74

1. Principal Investigator (give title and degrees):
Stephen F. Vatner, B.A., M.D.
Assistant Professor of Medicine, Harvard Medical School and Associate in Medicine, Peter Bent Brigham Hospital.

2. Institution & address:
Peter Bent Brigham Hospital
721 Huntington Avenue
Boston, Massachusetts 02115

3. Department(s) where research will be done or collaboration provided:
Department of Medicine, Peter Bent Brigham Hospital

4. Short title of study:
Nicotine induced reflex coronary vasodilation.

5. Proposed starting date: July 1, 1974

6. Estimated time to complete: 3 years.

7. Brief description of specific research aims:

1.) The effects of intravenous nicotine, 4-20 μg/kg, on the integrated control of heart rate, arterial pressure, myocardial contractility, cardiac output, total peripheral resistance and blood flow and resistance in the coronary, mesenteric, renal and iliac beds will be determined.

2.) The extent to which intravenous nicotine increases coronary blood flow and reduces coronary vascular resistance will be determined. The extent to which nicotine induced coronary vasodilatation is primary or secondary to changes in myocardial O_2 requirements will be examined by comparing effects of intravenous and intracarotid nicotine on coronary dynamics a) when the heart is in spontaneous rhythm, b) with heart rate controlled by pacing and c) after inotropic effects are blocked with propranolol, 1 mg/kg. In addition, arterial and coronary sinus oxygen will be measured to determine if coronary A-VO_2 difference narrows.

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3.) The efferent autonomic pathways involved in the changes in heart rate, total peripheral resistance and resistances in the coronary, mesenteric, renal and iliac beds will be determined by selective and combined blockades of beta adrenergic receptors with propranolol, 1 mg/kg, alpha adrenergic receptors with phentolamine, 1 mg/kg and cholinergic receptors with atropine, 0.2 mg/kg.

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4.) The extent to which nicotine induced circulatory changes, and the induced coronary dilatation in particular, are due to nicotine's chemoreceptor stimulating action will be determined by injecting 0.2-0.4 μg/kg nicotine through catheters previously implanted in the common carotid artery just proximal to the carotid sinus.

5.) The extent to which hyperventilation, through stimulation of pulmonary stretch receptors, resulting from nicotine's stimulation of chemoreceptors, regulates the circulation and specifically causes coronary dilation will also be examined by hyperinflating the lungs in conscious animals. The effects of pulmonary hyperinflation on heart rate, arterial pressure, myocardial contractility, cardiac output, total peripheral resistance and blood flow and resistance in the coronary, mesenteric, renal and iliac beds will be examined.

6.) The efferent pathways involved in the reflex pathways described in specific aims #4 and #5 will be examined as outlined in specific aim #3 above.

7.) Whether cigarette smoke can elicit coronary vasodilation in the conscious animal will also be determined by examining the responses of coronary blood flow, arterial pressure and left ventricular function to cigarette smoke inhaled through a tracheostomy.

8.) The above responses, specific aims #1-5, will be compared in the same animals after general anesthesia to demonstrate the importance of conducting this line of research in the conscious animal and to determine how general anesthesia modifies this aspect of reflex control of the circulation.

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8. Brief statement of working hypothesis:

Preliminary work in our laboratory indicates that intravenous nicotine produces striking coronary vasodilatation in the conscious animal (Fig. 1). The goal of this study is to delineate the mechanism by which this coronary vasodilation occurs. Specifically, it is proposed to 1) demonstrate that the nicotine induced coronary vasodilation is reflex in nature and 2) establish the afferent and efferent pathways involved in this response. It is known that nicotine stimulates chemoreceptors (1, 2), which in turn elicit hyperventilation (1, 2). It is proposed that the resultant hyperventilation, through stimulation of the lung inflation reflex (3-8), is responsible for the coronary vasodilatation. Finally, the extent to which this aspect of reflex control of the coronary circulation stimulated by nicotine is applicable to the rest of the circulation, i.e., left ventricular function, cardiac output, regional blood flows and regional vascular resistances will be examined.

9. Details of experimental design and procedures (append extra pages as necessary)

BACKGROUND AND RATIONALE

Existing knowledge regarding the cardiovascular effects of nicotine is based primarily on experiments performed in isolated organs or in open chest, anesthetized animals (9, 10). However, general anesthesia affects the control of the circulation exerted by the central nervous system to a variable extent and alters every aspect of circulatory function including heart rate, the level of myocardial contractile state (11, 12), vascular resistance (13) and in particular, the coronary vascular bed (14) and the distribution of regional blood flows (12, 15). Therefore, responses to normal physiological stimuli and to commonly used pharmacological agents may be quite different in normal healthy animals than in anesthetized animals or isolated organs. The applicants have demonstrated in conscious animals important differences from traditionally held concepts derived from studies in anesthetized animals, in diverse areas such as baroreceptor control of the circulation (16, 17), the influence of increasing cardiac frequency on myocardial contractility (Bowditch phenomenon) (18), increasing afterload on cardiac function (Anrep effect) (19), hemorrhage (20), the effects of catecholamines (21-23) and cardiac glycosides (11, 24, 25).

It is of particular importance to study the effects of nicotine on the coronary circulation in conscious animals, since nicotine exerts a variety of effects on the cardiovascular system (9, 10). It has direct effects on peripheral vessels, liberates catecholamines from adrenergic receptors and the adrenal medulla, stimulates the chemoreceptors, autonomic ganglia and stimulates myocardial contractility and myocardial metabolism. Despite the numerous investigations on the cardiovascular effects of nicotine (9, 10), of chemoreceptor stimulation (1, 2) and of the pulmonary inflation reflex (3-8), the finding that nicotine through stimulation of chemoreceptor and secondarily, the pulmonary inflation reflex, causes striking coronary vasodilation, has not been reported. The goal of this study is to elicidate these effects of nicotine in the conscious animal model.

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METHODS OF PROCEDURE

1. Implantation of Transducers. Two general preparations will be employed. One series of mongrel dogs, 20-35 kg, will be instrumented for studies involving control of cardiac output, total peripheral resistance and the distribution of regional blood flows and resistances. Another series of dogs will be instrumented for studies involving control of the coronary circulation, left ventricle and myocardial contractility. All operations will be conducted using sterile

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surgical technique and pentobarbital, Na 30 mg/kg for anesthesia. The first series of dogs will be instrumented through a left thoracotomy, with Doppler ultrasonic or electromagnetic aortic flow probes on the ascending aorta for cardiac output, miniature pressure gauges in the aorta for arterial pressure and through a midline laparotomy with Doppler or electromagnetic flow transducers on the mesenteric, renal and external iliac arteries. The second series of dogs will be instrumented through a left thoracotomy with Doppler or electromagnetic flow transducers on the left circumflex artery, miniature pressure gauges in the left ventricle for pressure, ultrasonic dimension transducers on opposing endocardial surfaces of the left ventricle for diameter and stimulating electrodes on the left atrium to maintain heart rate constant, and a Tygon catheter in the aorta to sample blood pressure and for arterial blood gas determination. In several of these dogs at a subsequent operation, using a right thoracotomy approach, catheters will be implanted chronically in the coronary sinus for coronary venous samples and coronary A-VO_2 determinations and in the right atrium for right atrial pressure.

For the series of experiments involving the role of pulmonary inflation reflexes stimulated indirectly via nicotine induced chemoreflex stimulation, PE-90 catheters will be implanted in the common carotid arteries proximal to the carotid sinuses.

2. Instrumentation Technique.

A. Measurement of Blood Flow. The ultrasonic Doppler flowmeter as developed by Franklin (26, 27) utilizes the principle that ultrasound reflected from a moving target, e.g., blood cells, exhibits a shift in frequency proportional to the velocity of the target. Continuous sonic energy is directed diagonally into the blood stream and a part of this energy is reflected from moving blood cells. The shift in frequency caused by this process provides an average of the instantaneous velocity of the red cells and is determined by extracting the frequency difference between transmitted and reflected sonic waves. An attractive and important feature of this flow system is the accurate electrical zero reference which does not require the additional implantation of an occluder to determine zero blood flow. When blood flow ceases, there is no Doppler shift; accordingly, the received ultrasonic signal is identical to the transmitted signal indicating zero flow.

A measure of volume flow is extracted by knowledge of the average velocity and internal cross-sectional area of the vessel. The relationship between velocity, as measured by the Doppler flowmeter, and volume flow is linear as long as the cross-sectional area of the blood vessel within the transducer remains constant. This linear relationship between velocity and volume flow has been demonstrated repeatedly previously and confirmed by means of timed collections of blood flow (27). At autopsy, it has been observed consistently that the vessels are firmly adherent to the flow transducers through a fibrous scar which minimizes changes in the cross-sectional area of the blood vessel within the flow transducers. Additionally, the scale factor for the flow measurement remains stable since the connective tissue growth has had time to fix the transducer to the blood vessel [illegible].

An electromagnetic flowmeter is more appropriate for certain experiments. For instance, measurement of ascending aortic blood flow and in situations of significant reverse flow. In these experiments a square wave electromagnetic type flowmeter (Statham SP2200) will be employed.

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B. System for Measurement of Arterial and Left Ventricular Pressures. Arterial pressure will be measured by means of a miniature intravascular pressure gauge as developed by Van Citters and Franklin (28), which will be implanted into the thoracic or abdominal aorta through a 1-2 cm. longitudinal incision closed by interrupted sutures to measure arterial pressure or implanted through a stab wound in the apex of the left ventricle and closed by a purse string suture to measure left ventricular pressure (28). The pressure gauge[1] consists of a titanium diaphragm, 5 mm. in diameter, which has bonded on its inner surface a silicon element Wheatstone bridge, which provides high fidelity signals proportional to blood pressure. This unit is sealed and thus measures absolute pressure. The signal output of the pressure gauge is 30 mV for 300 mmHg pressure change when excited by 5 volts DC.

C. System for Measurement of Left Ventricular Diameter. A measurement of left ventricular diameter will be obtained by determining the acoustic transit time of 3 MHz ultrasound propagated between two piezoelectric crystals placed in opposition on the endocardial surfaces of the left ventricle. In operation, the dimension gauge as developed by Patrick and coworkers (29), provides electrical impulses to one of the crystals and senses the propagated ultrasound impulses received by the opposing crystal. Rectangular voltage pulses are generated, the duration of which are equivalent to the transit time of the acoustic impulses, and are time averaged to produce a voltage proportional to ventricular diameter.

3. Experimental Protocol

The experiments will be conducted with the animals in the conscious state. Two to four weeks after recovery from operation, when the animals are vigorous and healthy, and after the animals are trained and accustomed to the laboratory and its personnel.

A. Effects of Intravenous Nicotine. After control recordings at rest and with the dogs in the basal state, small doses of nicotine (40 ug/kg) will be injected intravenously as a bolus. The resultant effects of chemoreflex stimulation and accompanying hyperventilation will be examined on heart rate, arterial and left ventricular pressures, left ventricular dimensions, myocardial contractility, coronary and regional blood flows and resistances. The efferent mechanism will be analyzed by repeating this procedure after selective and combined blockades of beta adrenergic receptors with propranolol, 1 mg/kg, alpha adrenergic receptors with phentolamine, 1 mg/kg, and cholinergic blockade with atropine, 0.2 mg/kg. Studies on the coronary circulation and myocardial contractility will be conducted with heart rate maintained constant as well.

In order to separate chemoreflex and pulmonary inflation inputs, the experiments will be repeated on a separate day in the same animals during an infusion of succinylcholine, 2-4 mg/min, and with respiration controlled with a Harvard ventilator. Thus, the direct chemoreflex effects will remain intact and the indirect stimulation of pulmonary inflation reflexes will be eliminated. It is recognized that succinylcholine is not an anesthetic agent; accordingly, no procedures will be conducted that would not be well tolerated by the dogs in the conscious state with succinylcholine. Intubation will be performed after spraying the larynx with a topical anesthetic. This procedure has already been done in conscious man (30).

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B. Effects of Intracarotid Nicotine. In order to separate systemic effects of nicotine from its simulation of chemoreceptors, nicotine will be injected in a bolus into the implanted carotid arterial catheters in small doses (0.2-0.4 ug/kg). The effects of intracarotid nicotine will be examined on the same parameters as mentioned in section A. above; 1) in spontaneous rhythm, 2) with heart rate constant, 3) after selective and combined autonomic blockades and 4) after succinylcholine infusion and with ventilation held constant.

C. Direct Stimulation of Pulmonary Inflation Reflexes. The same animals will be studied on a separate day during succinylcholine infusion. After steady state control measurements are recorded, ventilation will be rapidly changed, both in frequency and volume. The effects of brief periods of pulmonary hyperinflation on heart rate, arterial and left ventricular pressures, left ventricular function and myocardial contractility and blood flows and resistances in the coronary, mesenteric, renal and iliac beds will be examined. In the experiments involving the coronary circulation it will be important to measure right atrial pressure as well. The efferent autonomic pathways of these responses will be studied by repeating the procedure after selective and combined autonomic blockades.

D. Effects of Anesthesia in Modifying the Effects of Nicotine on the Circulation. The experiments described above in subsections A and B will be repeated after general anesthesia with pentobarbital, Na 30 mg/kg. These experiments will demonstrate the importance of conducting research that involves an interaction between the respiratory and cardiovascular systems in conscious animals and will demonstrate the effect of general anesthesia in modifying this aspect of ventilatory reflex control of the circulation.

E. Effects of Cigarette Smoke. The effects of cigarette smoke administered through a tracheostomy will be examined on the circulatory measurements described in section "A" above, to determine if the same effects, particularly, coronary dilatation, can be elicited with inhaled cigarette smoke as well as intravenous or intracarotid nicotine.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

1600 sq. ft. of laboratory space is currently being utilized by the principal investigator and his colleagues for studies of circulatory function in conscious animals. This laboratory, as well as existing equipment including tape recorder, 8 channel oscillograph, and instruments for the measurement and radio telemetry of blood flow, pressure and dimensions will be available for this research. These projects will also utilize existing animal facilities at Harvard Medical School, Children's Hospital Research Building, and the New England Regional Primate Center.

11. Additional facilities required:

None.

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12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
S.F. Vatner, M.D.	25%	0
E. Braunwald, M.D.	5%	0
Technical		
T. Manders - Research Assistant	50%	$5,000
R. Peters - Electronics Technician	50%	5,000
Fringe Benefits, 13.65%		1,365
	Sub-Total for A	$11,365

B. Consumable supplies (by major categories)

1. Dogs - 50 at $50	2,500
2. Boarding for dogs	1,500
3. Surgical Supplies	1,500
4. Miniature pressure gauge - 5 at $500 each	2,500
5. Flow probes	2,000
6. Lab supplies	2,000
Sub-Total for B	12,000

C. Other expenses (itemize)

1. Travel	500
2. Art and photography	250
3. Publication costs	250
4. Equipment maintenance	500
Sub-Total for C	1,500
Running Total of A + B + C	24,865

D. Permanent equipment (itemize)

1. Arterial blood gas analyzer	3,000
Sub-Total for D	3,000
E. Indirect costs (15% of A+B+C)	3,730
Total request	31,595

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	12,502	13,500	2,000	0	4,200	32,202
Year 3	13,752	15,000	2,000	0	4,613	35,365

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1) Cardiovascular control in normal and disease states. E. Braunwald, M.D., Principal Investigator.	1 RO1 HL15416-02 - N.I.H.	$92,240	1/1/73 - 12/31/77
2) Cardiovascular regulation in conscious animals.	Established Investigator - American Heart Association	-	July, 1974 - June, 1979
3) Effects of nicotine and cigarette smoke on coronary dynamics, blood flow distribution and myocardial contractility in conscious dogs. S.F. Vatner,M.D. Principal Investigator.	American Medical Association Grant # 407	$19,985	Nov.1,1972 - Oct.31,1974
4) Cardiovascular Control mechanism in conscious baboons. S.F. Vatner, M.D., Principal Investigator.	American Heart Association	$16,000 1st year	7/1/73 - 6/30/76

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Stephen F. Vatner, M.D.

Signature Stephen F. Vatner Date 11/31/74

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Responsible officer of institution

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Title Director, Peter Bent Brigham Hopsital

Signature William E Hassan Jr Date 11/31/74

Telephone 617 (Area Code) 734-8000 (Number) 2101 (Extension)

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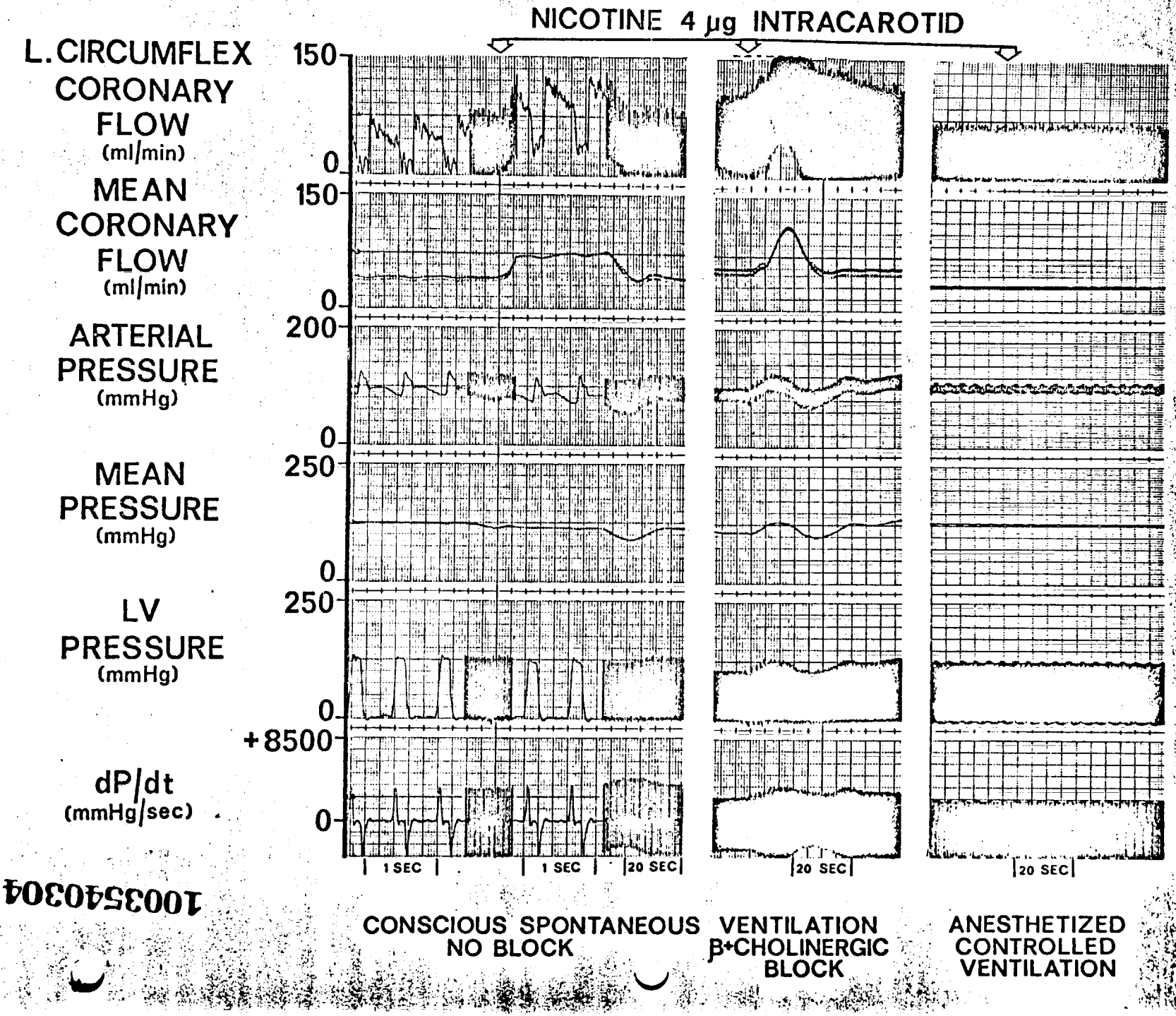

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Figure 1: Responses to intracarotid nicotine, 4 ug, in the same dog, conscious and with spontaneous ventilation (left panel), after beta receptor and cholinergic blockades (middle panel), and with controlled ventilation after pentobarbital, Na anesthesia (right panel). Responses of phasic and mean left circumflex coronary flow, phasic and mean arterial pressure, left ventricular pressure and dP/dt are shown. Note the striking increase in coronary flow associated with chemoreceptor stimulation and hyperventilation in the conscious animal (left and middle panels) and the absence of virtually any response at this dose of nicotine in the same animal after anesthesia (right panel).

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